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NMDA-induced glutamate and aspartate release from rat cortical pyramidal neurones: evidence for modulation by a 5-HT_{1A} antagonist

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1 We have investigated an aspect of the regulation of cortical pyramidal neurone activity. Microdialysis was used to assess whether topical application of drugs (in 10 μl) to fill a burr hole over the frontal cortex, where part of the corticostriatal pathway originates, would change concentrations of the excitatory amino acids glutamate and aspartate in the striatum of the anaesthetized rat.

2 Topical application of N-methyl-D-aspartate (NMDA, 2 and 20 mM) dose-dependently increased glutamate and aspartate concentrations in the striatum. Coapplication of tetrodotoxin (10 μM) blocked the NMDA-evoked rise in these amino acids. A calcium-free medium, perfused through the probe also blocked the rise, indicating that it was due to an exocytotic mechanism in the striatum.

3 It was hypothesized that the rise observed was due to an increase in the activity of the corticostriatal pathway. As 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}) receptors are enriched on cell bodies of corticostriatal neurones, a selective 5-HT_{1A}-antagonist (WAY 100135) was coapplied with the lower dose of NMDA. Compared to NMDA alone, coapplication of 50 μM WAY 100135 significantly increased glutamate release. This effect was sensitive to tetrodotoxin and calcium-dependent. Application of 50 μM WAY 100135 alone significantly enhanced glutamate release above baseline; this was also tested at 100 μM (not significant).

4 Compared to NMDA alone, coapplication of WAY 100135 (20 μM) significantly enhanced aspartate release; the mean value was also increased (not significantly) with 50 μM. This rise was calcium-dependent, but not tetrodotoxin-sensitive. WAY 100135 (100 μM) reduced NMDA-induced aspartate release. Application of the drug alone had no effect on basal aspartate release.

5 Coapplication of the 5-HT_{1A} agonist, 8-OHDPAT (5 mM) with NMDA did not affect the NMDA-evoked increase in glutamate and aspartate.

6 Topical application of high potassium (100 mM) to the surface of the cortex did not result in a detectable rise in striatal glutamate or aspartate.

7 Perfusion of WAY 100135 (tested at 50 μM) through the dialysis probe did not affect glutamate or aspartate concentrations.

8 It was concluded that a selective 5-HT_{1A}-antagonist can increase the activity of corticostriatal pyramidal neurones. As in Alzheimer's disease hypoactivity of pyramidal neurones almost certainly exists, a selective 5-HT_{1A}-antagonist may be potentially useful in the treatment of the cognitive symptoms of this disease.

Keywords: Alzheimer's disease; WAY 100135; microdialysis; excitatory amino acids; cortex; striatum; cortical pyramidal neurone; 5-HT_{1A}-antagonist

Introduction

Pyramidal neurones constitute the most abundant neuronal type in the mammalian neocortex (Winfield *et al.*, 1980). Loss and hypoactivty of cortical pyramidal neurones may underlie the cognitive impairment in Alzheimer's disease. This may be exacerbated by reduced excitatory modulation of pyramidal neurones by acetylcholine and maintained inhibitory tone through 5-HT_{1A} receptors (Francis *et al.*, 1993b).

We have previously shown that chemical stimulation of the neocortex of anaesthetized rats with N-methyl-D-aspartate (NMDA), in combination with a cocktail of γ-aminobutyric acid (GABA) antagonists, increased the extracellular concentration of glutamate and aspartate in the striatum as measured by microdialysis with h.p.l.c. (Palmer *et al.*, 1989). This is considered to reflect increased firing of layer V pyramidal neurones which form the corticostriatal pathway. Several studies have shown a modulatory action of 5-hydroxytryptamine (5-HT) on the responses of excitatory amino acids in the mammalian nervous system, with both suppressive and enhancing effects reported (McCall & Aghajanian, 1979; Lee *et al.*, 1986; Reynolds *et al.*, 1988). This divergence may be explained by the subtypes of 5-HT receptors present on the neurones under study, in that activation of different receptor subtypes could produce either neuronal inhibition or excitation. Other studies indicate that corticostriatal pyramidal neurones of layer V of the rat are enriched with 5-HT_{1A} receptors (Pangalos *et al.*, 1991, Francis *et al.*, 1992). Pyramidal neurones are hyperpolarized by 5-HT through the 5-HT_{1A} receptor (Andrade & Nicholl, 1987; McCormick & Williamson, 1989).

The working hypothesis of this study was therefore that a selective 5-HT_{1A} antagonist would block the hyperpolarizing effect of endogenous 5-HT on pyramidal neurones in layer V of the rat cortex, and would potentiate the effect of a depolarizing agent such as NMDA.

Until recently only partial agonists of the 5-HT_{1A} receptor were available. However, recently a selective antagonist, WAY 100135 (N-*tert*-butyl 3-4(2-methoxyphenyl) piperazin 1-yl-2-phenylpropanamidedihydrochloride) was described. This is an

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antagonist in both pre and post-synaptic 5-HT$_{1A}$ models and is devoid of agonist properties up to 10 mg kg^{-1}. (Fletcher *et al.*, 1993; Routledge *et al.*, 1993). Since the activity of WAY 100135 resides in the (+)-enantiomer, (+)-WAY 100135 was used for all experiments.

The long-term aim of this study is to gather evidence that 5-HT$_{1A}$ antagonists may be of potential benefit for the symptomatic treatment of cognitive deficits in Alzheimer's disease patients (Francis *et al.*, 1993b).

Methods

Animals and surgery

Male Sprague Dawley rats (Charles River, U.K.) weighing between 200 and 250 g were housed individually and had free access to food and water with a 12 h light/dark cycle (lights on 07 h 00 min). Humidity and temperature were maintained at 45–50% and 20–21°C, respectively. Before the experiments animals were allowed a one week period of adaptation. At the start of the experiment rats were anaesthetized with halothane in a mixture of oxygen and nitrous oxide until no reaction to tail and hind paw pinch was detectable. Under full anaesthesia animals were mounted in a Kopf stereotactic frame and a craniotomy was made over the frontal cortex by removal of a bone flap and the dura. A second burr hole was made over the striatum to allow implantation of the microdialysis probe (see microdialysis section). Drugs were applied with a 10 μl Hamilton syringe (Hamilton Bonaduz AG, Switzerland).

Microdialysis

A concentric microdialysis probe (membrane length 4 mm., diameter 200 μm) was implanted in the striatum (coordinates with bregma as reference: AP 0 mm, L 2.5 mm, DV 7 mm from skull). The microdialysis probe used was based on those described previously (Hutson *et al.*, 1985) except that internal glass capillary tubes were replaced by fused silica tubing (0.19 mm o.d.; 0.075 mm i.d.; SGE Ltd, Milton Keynes, Beds.) and membrane material was copolymer of acrylonitrile and sodium methallyl sulphonate (AN 69; 0.31 mm OD, 0.22 mm ID; Hospal Medical, New Brunswick, N.Y., U.S.A.).

The probe was perfused with a modified phosphate buffered saline; mPBS (PBS tablets; Sigma Chemical Co., Poole, Dorset) containing in mM: NaCl 137, $CaCl_2$ 1.3, $MgCl_2$ 1.3, KCl 5, (pH = 7.3; mPBS) at 1 μl min^{-1}, with a Carnegie Medicine micro-infusion pump.

To determine dead volume and *in vitro* recovery probes were placed in 40 ml mPBS containing glutamate and aspartate (50 μM) at 37°C. Nine (collection time: 10 min) samples were collected and analysed for glutamate and aspartate concentration using h.p.l.c. (Palmer *et al.*, 1989).

In the series where calcium-dependency of the effects were studied, the probe was perfused with a calcium-free medium containing in mM: NaCl 138.9, $MgCl_2$ 1.3, KCl 5, (pH = 7.3; mPBS) at 1 μl min^{-1}. Perfusion was started from the moment of implantation.

Experimental design

After implantation of the microdialysis probe and a stabilisation period of 1 h, 6 baseline samples were collected (collection time per sample: 10 min). At the beginning of each collection period the craniotomy over the frontal cortex was filled with 10 μl of vehicle (mPBS). At the beginning of the seventh sample the craniotomy was filled with 10 μl of drugs dissolved in mPBS. A further eight samples were collected. At the beginning of each collection period the craniotomy was filled with vehicle. Throughout the experiment core temperature was kept at a constant 37.7°C with a heating pad linked to a automated feedback monitoring device (Carnegie Medicine, Stockholm, Sweden).

The following drugs (in a volume of 10 μl) were applied to the frontal cortex: (A) vehicle (mPBS) $n=8$; (B) NMDA (2 mM, $n=7$; 20 mM, $n=6$); (C) NMDA (20 mM), coapplied with tetrodotoxin 10 μM ($n=5$); (D) NMDA (2 mM), coapplied with WAY 100135 20 μM ($n=4$), 50 μM ($n=13$), 100 μM ($n=7$); (E) NMDA (2 mM), coapplied with 8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT, 5 mM; $n=7$); (F) NMDA (2 mM), coapplied with WAY 100135 50 μM together with tetrodotoxin 10 μM ($n=5$); (G) WAY 100135 topically applied (50 μM, $n=10$; 100 μM, $n=10$); WAY 100135 (50 μM) perfused through the probe ($n=4$); (H) NMDA (20 mM), dialysis with calcium-free medium ($n=8$); (I) NMDA (2 mM) + WAY 100135 (50 μM), dialysis with calcium-free medium ($n=7$); (J) 100 mM potassium ($n=4$).

H.p.l.c. analysis for aspartate and glutamate

Samples were stored at −70°C until analysed for glutamate and aspartate concentration by h.p.l.c. following precolumn derivatisation with *o*-phthaldialdehyde (Lowe *et al.*, 1990). Briefly, the solution used to form fluorescent derivatives of the amino acids was prepared each week by adding 27 mg *o*-phthaldialdehyde (Sigma Chemical Co., Poole, Dorset) dissolved in 0.5 ml 100% ethanol) to 5 ml of borate buffer (0.1 M) containing 10 μl of mercaptoethanol. This solution was protected from light and 50 μl added to either 10 μl of a standard mixture or 10 μl of dialysate sample. This mixture was allowed to react for exactly 2 min before application to the column. The h.p.l.c. apparatus consisted of a solvent delivery system (2 LKB Bromma 2150 h.p.l.c. pumps), a gradient controller (Aston LC, Aston Scientific, Aston Clinton, Bucks), injection valve with 20 μl loop (Model 7125 Rheodyne Inc., Cotati, CA, U.S.A.), a reverse phase octadecylsilane column of particle size 3 μm 10 cm by 4.6 mm (Ultratechsphere 3 ODS, h.p.l.c. Technology, Macclesfield, Cheshire), together with a fluorometric detector (LDC Fluoromonitor; excitation 370 nm filter; emission 418-700 nm wavelength band pass). The eluent was a mixture of 0.05 M sodium phosphate buffer (pH = 5.7) and methanol in the vol/vol proportion of 80:20 for solvent A and 20:80 for solvent B. A multilinear gradient was used (95% A, changing to 15% over 18 min and returning to 95% over 3 min); the flow rate was 1 ml min^{-1}. Peaks were quantified by measuring peak areas. Chemicals used were of the highest grade available (Sigma Chemical Co., Poole, Dorset).

Statistics

Values are expressed as mean pmol amino acids 10 μl^{-1} dialysate ± s.e.mean, and were not corrected for recovery across the membrane. Means of the six baseline samples for all groups were compared *post-hoc* by Kruskal-Wallis ANOVA. The effect of drugs on the concentration of glutamate and aspartate in the eleventh sample (maximal effect) was compared with that of the vehicle by Kruskal-Wallis ANOVA. Differences between means of drug groups of particular interest were compared by the Mann-Whitney-U test. The null hypothesis was rejected at $P<0.05$.

Results

In vitro recovery of probes ($n=5$) was 32 ± 5.6% (mean ± s.d.) for glutamate and 35 ± 7.5% for aspartate.

There were no significant differences in the concentration of glutamate or aspartate in baseline samples between treatment groups ($P>0.05$, Kruskal-Wallis ANOVA). The maximal effect of topical application of drugs on the concentration of glutamate and aspartate was consistently seen in the eleventh sample.

In vitro experiments show that the delay of the microdialysis system is between 5 and 10 μl (data not shown).

Figure 1 shows the effect of topical application to the frontal cortex of NMDA (20 mM) on striatal concentrations of

aspartate (a) and glutamate (b). NMDA increased significantly the concentration in the striatal dialysate of both amino acids, as compared to vehicle (aspartate: vehicle = 0.8 ± 0.1; NMDA = 11.4 ± 2.5) (glutamate: vehicle = 2.6 ± 0.4; NMDA = 12.0 ± 2.4). The effect on aspartate was sensitive to tetrodotoxin (TTX), which reduced the response to 3.1 ± 1.5. Calcium-free medium perfused through the probe reduced the effect to 3.2 ± 0.4. Similarly, NMDA induced glutamate release was sensitive to TTX (reduction of the effect to 3.9 ± 1.8). Calcium-dependency was also observed (reduction to 6.3 ± 1.5).

Figure 2 shows the effect of the 5-HT$_{1A}$ antagonist WAY 100135 on the NMDA (2 mM)-induced rise in aspartate (a) and glutamate (b). For clarity only the eleventh sample (sample where the maximal effect was consistently seen) is shown. Coapplication of WAY 100135 (20 μM) with NMDA significantly enhanced aspartate release, compared to NMDA alone (from 6.2 ± 0.4 to 9.3 ± 0.6). Coapplication of WAY 100135 (50 μM) did not significantly affect aspartate release but 100 μM WAY 100135 reduced the effect to 1.1 ± 0.2. Application of WAY 100135 50 μM alone had no effect on basal aspartate release.

Compared to NMDA alone, coapplication of 50 μM WAY 100135 (but not 20 or 100 μM) significantly increased glutamate release from 7.8 ± 0.9 to 14.3 ± 1.8. Application of WAY 100135 (50 μM) alone significantly enhanced baseline glutamate release from 2.6 ± 0.4 to 3.8 ± 0.3.

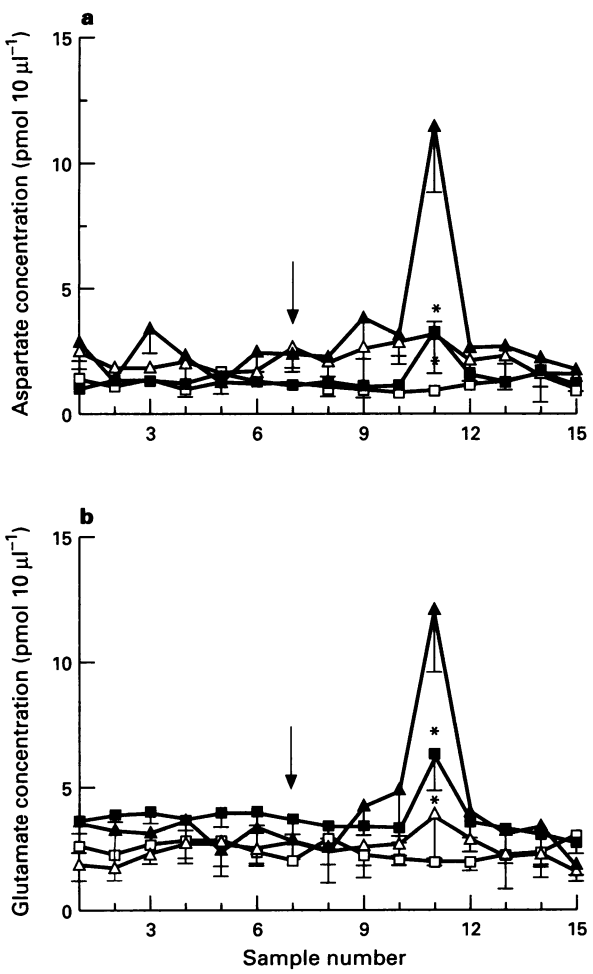

Figure 1 Effect of NMDA (▲, 20 mM, $n=6$), topically applied to the frontal cortex at the beginning of sample seven (arrow), vehicle (□, modified phosphate buffered saline, $n=8$) and NMDA (20 mM) in combination with tetrodotoxin (△, 10 μM, $n=5$) on the concentration of aspartate (a) and glutamate (b) in striatal dialysate. Also calcium-dependency of NMDA (20 mM; ■, $n=8$) is given. Data are presented as mean with s.e.mean. *Denotes difference from the NMDA effect (*$P<0.05$; Mann Whitney-U test).

8-OHDPAT (5 mM) coapplied with NMDA 2 mM did not significantly affect the release of aspartate or glutamate caused by NMDA alone (7.8 ± 0.9 versus 8.8 ± 1.0 for glutamate; 6.2 ± 0.4 versus 6.3 ± 1.3 for aspartate; data not shown).

Figure 3 shows TTX-sensitivity and calcium-dependency of the NMDA (2 mM) + WAY 50 μM-induced rise in (a) aspartate and (b) glutamate. The rise in aspartate was not sensitive to TTX but calcium-free medium reduced the rise from 9.4 ± 1.2 to 3.2 ± 0.8. The rise in glutamate was reduced by TTX from 14.3 ± 1.8 to 4.2 ± 0.5 and by calcium-free medium from 14.3 ± 1.8 to 6.7 ± 1.7.

Discussion

The present study investigates the effect of topically applied drugs on the activity of the frontal cortex, using striatal aspartate and glutamate release as a parameter of activity. The concept of excitatory amino acid neurotransmission is now well-established (Fagg & Foster, 1983; Cotman *et al.*, 1987)

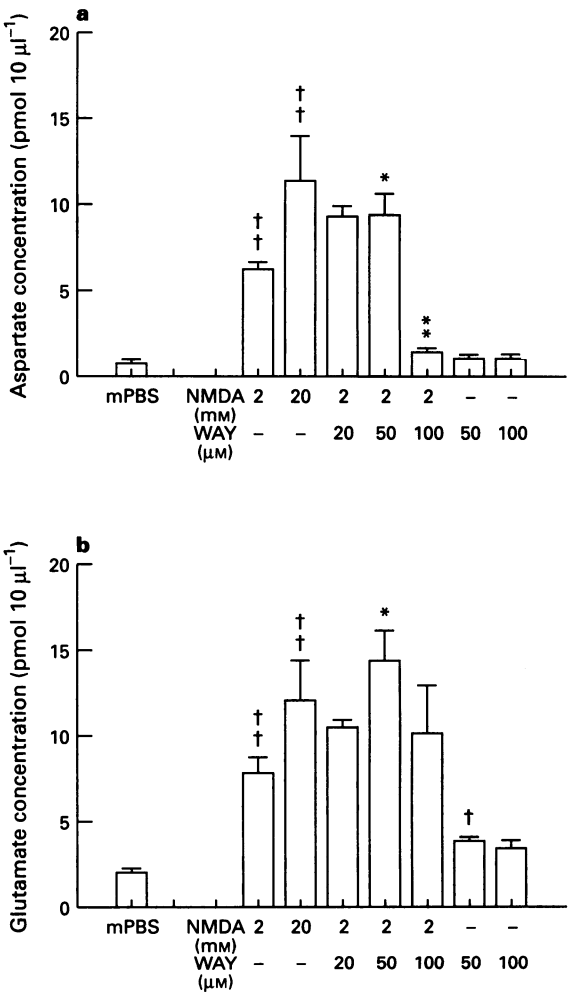

Figure 2 Effects of drugs topically applied to the frontal cortex on striatal release of aspartate (a) and glutamate (b) in the eleventh sample, measured by microdialysis and h.p.l.c. The effects illustrated are: vehicle (mPBS, $n=8$), NMDA (2 mM, $n=7$; 20 mM, $n=6$), NMDA (2 mM) coapplied with WAY 100135 (20 μM, $n=4$; 50 μM, $n=13$; 100 μM, $n=7$) and WAY 100135 alone (50 μM, $n=10$; 100 μM, $n=10$). Columns represent mean with s.e.mean. For clarity only the eleventh sample, where the maximal effect was consistently seen, is shown. †Denotes difference from the vehicle effect; Kruskall Wallis ANOVA, Mann Whitney U-test (†$P<0.05$; ††$P<0.01$); *denotes difference from the NMDA effect (*$P<0.05$; **$P<0.01$; Mann Whitney-U test).

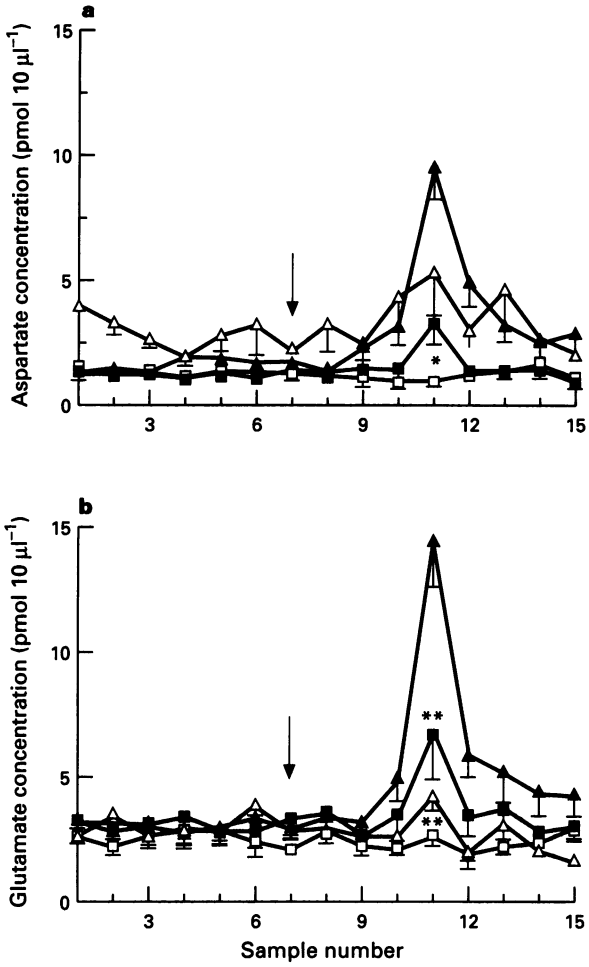

Figure 3 Effects of NMDA (2 mM) + WAY 100135 (50 µM) (▲, $n=13$), topically applied to the frontal cortex at the beginning of sample seven (arrow), vehicle (□, modified phosphate buffered saline, $n=8$) and combination of the two drugs with tetrodotoxin (△, 10 µM, $n=5$) on the concentration of aspartate (a) and glutamate (b) in striatal dialysate. Also calcium-dependency of the NMDA + WAY effects (■, $n=7$) is given. Data are presented as mean with s.e.mean. *Denotes difference from the NMDA effect (*$P<0.05$; **$P<0.01$; Mann Whitney-U test).

and the two amino acids L-aspartate and L-glutamate satisfy most of the criteria for neurotransmitter status (Orrego, 1979). For example, aspartate and glutamate are released in a calcium-dependent manner by electrical and chemical depolarization of neurones in various brain regions (Fonnum, 1984; Nicholls, 1989).

That both aspartate and glutamate serve a neurotransmitter function is suggested by the fact that topically applied NMDA caused a rise in both striatal aspartate and glutamate, which was dependent on coapplied tetrodotoxin, an effect not previously investigated (Palmer *et al.*, 1989). This suggests that depolarization of the cortex and more specifically of pyramidal neurones in layer V of the cortex, underlies the rise in these striatal amino acids. In addition, omission of calcium from the perfusion fluid markedly reduced the effect of NMDA, and of the combination of NMDA and WAY 100135. The observation that the overflow of the two amino acids was sensitive to calcium suggests that the phenomenon observed is due to release from terminals of pyramidal neurones by exocytosis rather than by cytoplasmic release or decreased reuptake.

It could be argued that application of NMDA to the surface of the cortex would cause a spreading depression of Leao, characterized by loss of cellular ionic homeostasis and a drastic redistribution of ions between the extracellular and intracellular compartments (Leao, 1944), which would then spread through to the deeper layers. Thus, increased release of amino acids could be the result of a nonspecific effect, rather than being the result of an interaction between drugs applied and receptors on the cell bodies of layer V pyramidal neurones. The main reason that this is unlikely is the delay of approximately 30 to 40 min observed between application of drugs and the maximal release of neurotransmitters in the striatum. *In vitro* experiments show that the delay cannot be attributed to the dead volume of the microdialysis system. In addition, spreading depression is not mediated by voltage-gated sodium channels (Tobiasz & Nicholson 1982; Aitken *et al.*, 1991), but in the present study, tetrodotoxin significantly reduced the effect of topically applied drugs. Furthermore a previous study using this paradigm showed that *infusion* of drugs into the frontal cortex had a similar effect to topical application of the drugs to the same frontal region (Palmer *et al.*, 1989). Application of 100 mM potassium to the surface of the cortex, did not affect striatal concentrations of either aspartate or glutamate. The rationale behind the experiments with 100 mM K^+ is the difference in diffusibility into brain tissue between NMDA and potassium. While potassium will be quickly taken up both by neurones and glial cells, NMDA will be taken up only at a low rate by low-affinity transport (Skerrit & Johnston, 1981; Garthwaite, 1985). Thus it can be postulated that although both potassium and NMDA will depolarize the surface of the cortex when applied topically, only NMDA will be able to diffuse to layer V of the cortex to depolarize directly the pyramidal neurones which form the corticostriatal pathway. This diffusion will take a certain amount of time, which may explain the delay seen in the present experiments. The reason why both NMDA and potassium, when applied topically fail to depolarize pyramidal neurones in layer V of the cortex immediately remains unclear. One possibility is that both excitatory and inhibitory neurotransmitters are released with no net effect on layer V.

Another explanation for the overflow of amino acids is that drugs applied to the cortex diffuse into the striatum and cause an increase in neurotransmitter release by *direct* interaction with receptors on corticostriatal terminals. Three observations make this unlikely; no NMDA was detected in the striatal microdialysate (Palmer *et al.*, 1989) and secondly, there is no evidence for the existence of presynaptic NMDA receptors on terminals of the corticostriatal pathway (Greenamyre & Young, 1989). The third observation is that, although topical application of WAY 100135 to the frontal cortex gave a rise in glutamate, perfusion of the drug through the probe had no effect. In conclusion, the most likely reason for the observed effects of the topically applied drugs is that they diffuse through the cortex and only given an effect on striatal glutamate and aspartate release when the cell bodies of the neurones in layer V of the cortex are directly depolarized.

NMDA-induced release of glutamate and aspartate was potentiated by the 5-HT$_{1A}$ antagonist, WAY 100135, although at different concentrations. The most straightforward explanation for this effect is that the compound reduces the resting potential of cortical pyramidal neurones, by blocking the action of 5-HT, an hyperpolarizing transmitter, thereby increasing the likelihood that a given cell is depolarized by NMDA. Pioneering (Krnjevic & Phillis, 1963), and more recent (Andrade & Nicholl, 1987; Colino & Halliwell, 1987; Araneda & Andrade, 1991) electrophysiological studies suggest an overall inhibitory action of 5-HT on the cortex, a hypothesis supported by this study. Therefore, the observation of Ceci *et al.* (1993) that peripherally administered fluoxetine *increased* overall cortical activity in the rat was unexpected. However, the authors explain this by citing a microdialysis study where fluoxetine preferentially increased 5-HT concentrations in the dorsal raphe, the source of the 5-HT innervation to the cortex. Presynaptic 5-HT$_{1A}$ receptors, located on the neuronal bodies in the dorsal raphe would therefore be preferentially activated by fluoxetine resulting in a lowering of 5-HT concentrations in the cortex, resulting in disinhibition.

That WAY 100135 acts as an antagonist under the present experimental conditions is suggested by the results obtained with the 5-HT$_{1A}$ agonist, 8-OHDPAT, which failed to affect the NMDA response. This could be due to the fact that endogenous levels of 5-HT saturate the endogenous 5-hydroxytryptaminergic modulation. Under these circumstances the effects of WAY 100135 cannot be ascribed to an agonist effect.

An unexpected divergence in the response of aspartate and glutamate to WAY 100135 was clearly evident (compare Figure 2a with 2b). Although the effect of the lower concentration of NMDA on both aspartate and glutamate was potentiated by WAY 100135, the maximum potentiation of aspartate release was at a lower concentration of the drug (20 μM) than for the effect on glutamate (50 μM). Similarly, WAY 100135 (100 μM) did not alter NMDA-induced glutamate release but significantly attenuated NMDA-induced aspartate release compared to NMDA alone. There is no clear explanation for the divergent effects of WAY 100135 on aspartate and glutamate release. One possibility is that the corticostriatal pathway consists of at least two types of neurone, one using aspartate preferentially as a transmitter the other, glutamate. Such neurone populations may have 5-HT$_{1A}$ receptors which are differently coupled to an effector system. This could result in a different pharmacological profile, an idea consistent with the detection of three mRNAs in the mammalian brain, suggesting the existence of subtypes of 5-HT$_{1A}$ receptors (Albert *et al.*, 1990). Alternatively, there may be a greater enrichment of 5-HT$_{1A}$ receptors on putative aspartergic compared with glutamatergic neurones.

The status of the 5-HT system in Alzheimer's disease is complicated. Reports of reduced 5-HT concentrations in postmortem studies of Alzheimer's disease may have been complicated by increased 5-HT turnover (Palmer *et al.*, 1987a,b; Sparks *et al.*, 1992; Francis *et al.*, 1993a) and biased by the unintentional selection of subjects for whom institutional care was necessary. Indeed, reduced 5-HT in orbitofrontal cortex was observed only in Alzheimer's disease patients with aggressive behaviour (Palmer *et al.*, 1988). A recent community-based study of Alzheimer's disease patients found a substantial reduction in numbers of dorsal raphe neurones, but in orbitofrontal and temporal cortex there was less marked loss of binding of [^{3}H]-paroxetine to the reuptake site and no significant reduction in 5-HT (Chen *et al.*, 1994). This was accompanied by evidence of increased 5-HT turnover. The 5-HT$_{1A}$ receptor has been shown to be fully functional in *post-mortem* Alzheimer's disease brain (O'Neill *et al.*, 1991) and is almost certainly preferentially located on cortical pyramidal neurones in layer II of human cortex (DeFelipe & Jones, 1988; Bowen *et al.*, 1992; Barone *et al.*, 1994). These data, taken together with the hyperpolarizing effect of such receptors indicates the maintenance of an 'inhibitory tone' on the remaining cortical pyramidal neurones in Alzheimer's disease. This action would exacerbate the loss of excitatory modulation due to glutamatergic hypoactivity and cholinergic dysfunction which may underlie the cognitive symptoms of the disease (Francis *et al.*, 1993b).

The present study indicates that selective 5-HT$_{1A}$-antagonists can potentiate the effect of a depolarizing agent (NMDA) on the activity of glutamatergic pyramidal neurones as well as facilitate endogenous neurotransmission in the rat and may therefore be useful for the symptomatic treatment of patients with Alzheimer's disease. In addition, evidence is emerging that increasing activity of these neurones may reduce the formation of senile plaques and neurofibrillary tangles, histological hallmarks of the disease (Bowen *et al.*, 1994).

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